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*November 22, 2004*

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**APPLICATION NUMBER:** *10/686,904*
**FILING DATE:** *October 16, 2003*
**RELATED PCT APPLICATION NUMBER:** *PCT/US04/34605*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

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PTO/SB/05 (03-01)
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# UTILITY PATENT APPLICATION TRANSMITTAL

*(Only for new nonprovisional applications under 37 C.F.R. 1.53(b))*

| | |
|---|---|
| Attorney Docket No. | 010023-001200US |
| First Inventor | John H. Crowe, et al. |
| Title | BIOLOGICAL SAMPLES AND METHOD FOR STABILIZING BIOLOGICAL SAMPLES |
| Express Mail Label No. | EU121945984US |

2386 U.S. PTO 10/696004 10/16/03

## APPLICATION ELEMENTS

*See MPEP chapter 600 concerning utility patent application contents.*

1. ☒ Fee Transmittal Form (e.g., PTO/SB/17) *(Submit an original and a duplicate for fee processing)*
2. ☒ Applicant claims small entity status. See 37 CFR 1.27.
3. ☒ Specification [Total Pages 57 ] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross Reference to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to sequence listing, a table, or a computer program listing appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *( if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
4. ☒ Drawing(s) (35 U.S.C.113) [Total Sheets 4 ]
5. Oath or Declaration [Total Pages 0 ]
   a. ☐ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63 (d)) *(for a continuation/divisional with Box 18 completed)*
      i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
6. ☐ Application Data Sheet. See 37 CFR 1.76
7. ☐ CD-ROM or CD-R in duplicate, large table or Computer Program *(Appendix)*
8. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. ☐ Computer Readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM or CD-R (2 copies); or
      ii. ☐ paper
   c. ☐ Statements verifying identity of above copies

## ADDRESS TO:

Assistant Commissioner for Patents
Box Patent Application
P.O. Box 1450
Alexandria, VA 22313-1450

## ACCOMPANYING APPLICATIONS PARTS

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 C.F.R.§3.73(b) Statement ☐ Power of Attorney *(when there is an assignee)*
11. ☐ English Translation Document *(if applicable)*
12. ☒ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
13. ☐ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
16. ☐ Non publication Request under 35 U.S.C. 122 (b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.
17. ☒ Other: Express Mail Certification

**18. If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment, or in an Application Data Sheet under 37 CFR 1.76:*

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: ____ / ____

*Prior application information:* *Examiner* ____ *Group / Art Unit:* ____

**For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.**

## 17. CORRESPONDENCE ADDRESS

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| Name (Print/Type) | John W. Carpenter | Registration No. (Attorney/Agent) | 26,447 |
| Signature | | Date | 10/15/03 |

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# FEE TRANSMITTAL for FY 2002

*Patent fees are subject to annual revision.*

**TOTAL AMOUNT OF PAYMENT** ($) 471.00

| *Complete if Known* | |
|---|---|
| Application Number | Unknown |
| Filing Date | October 15, 2003 |
| First Named Inventor | John H. Crowe, et al. |
| Examiner Name | Unknown |
| Group / Art Unit | Unknown |
| Attorney Docket No. | 010023-001200US |

## METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

Deposit Account Number

Deposit Account Name

☐ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17

☐ Applicant claims small entity status. See 37 CFR 1.27

2. ☒ Payment Enclosed:

☒ Check ☐ Credit card ☐ Money Order ☐ Other

## FEE CALCULATION

### 1. BASIC FILING FEE

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 101 | 740 | 201 | 370 | Utility filing fee | 385.00 |
| 106 | 330 | 206 | 165 | Design filing fee | |
| 107 | 510 | 207 | 255 | Plant filing fee | |
| 108 | 740 | 208 | 370 | Reissue filing fee | |
| 114 | 160 | 214 | 80 | Provisional filing fee | |

SUBTOTAL (1) ($)385.00

### 2. EXTRA CLAIM FEES

| | | | Extra Claims | | Fee from below | | Fee Paid |
|---|---|---|---|---|---|---|---|
| Total Claims | 19 | -20 = | 0 | X | 9 | = | 0 |
| Independent Claims | 5 | -3 = | 2 | X | 43 | = | 86 |
| Multiple Dependent | | | | X | | = | 0 |

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description |
|---|---|---|---|---|
| 103 | 18 | 203 | 9 | Claims in excess of 20 |
| 102 | 84 | 202 | 42 | Independent claims in excess of 3 |
| 104 | 280 | 204 | 140 | Multiple dependent claim, if not paid |
| 109 | 84 | 209 | 42 | ** Reissue independent claims over original patent |
| 110 | 18 | 210 | 9 | ** Reissue claims in excess of 20 and over original patent |

SUBTOTAL (2) ($) 471.00

**or number previously paid, if greater; For Reissues, see above

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 105 | 130 | 205 | 65 | Surcharge - late filing fee or oath | |
| 127 | 50 | 227 | 25 | Surcharge - late provisional filing fee or cover sheet. | |
| 139 | 130 | 139 | 130 | Non-English specification | |
| 147 | 2,520 | 147 | 2,520 | For filing a request for reexamination | |
| 112 | 920* | 112 | 920* | Requesting publication of SIR prior to Examiner action | |
| 113 | 1,840* | 113 | 1,840* | Requesting publication of SIR after Examiner action | |
| 115 | 110 | 215 | 55 | Extension for reply within first month | |
| 116 | 400 | 216 | 200 | Extension for reply within second month | |
| 117 | 920 | 217 | 460 | Extension for reply within third month | |
| 118 | 1,440 | 218 | 720 | Extension for reply within fourth month | |
| 128 | 1,960 | 228 | 980 | Extension for reply within fifth month | |
| 119 | 320 | 219 | 160 | Notice of Appeal | |
| 120 | 320 | 220 | 160 | Filing a brief in support of an appeal | |
| 121 | 280 | 221 | 140 | Request for oral hearing | |
| 138 | 1,510 | 138 | 1,510 | Petition to institute a public use proceeding | |
| 140 | 110 | 240 | 55 | Petition to revive – unavoidable | |
| 141 | 1,280 | 241 | 640 | Petition to revive – unintentional | |
| 142 | 1,280 | 242 | 640 | Utility issue fee (or reissue) | |
| 143 | 460 | 243 | 230 | Design issue fee | |
| 144 | 620 | 244 | 310 | Plant issue fee | |
| 122 | 130 | 122 | 130 | Petitions to the Commissioner | |
| 123 | 50 | 123 | 50 | Petitions related to provisional applications | |
| 126 | 180 | 126 | 180 | Submission of Information Disclosure Stmt | |
| 581 | 40 | 581 | 40 | Recording each patent assignment per property (times number of properties) | |
| 146 | 740 | 246 | 370 | Filing a submission after final rejection (37 CFR § 1.129(a)) | |
| 149 | 740 | 249 | 370 | For each additional invention to be examined (37 CFR § 1.129(b)) | |
| 179 | 740 | 279 | 370 | Request for Continued Examination (RCE) | |
| 169 | 900 | 169 | 900 | Request for expedited examination of a design application | |

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

## SUBMITTED BY

Complete *(if applicable)*

| | | | | | |
|---|---|---|---|---|---|
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| *Signature* | | | | *Date* | 10/15/03 |

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| | |
|---|---|
| **In Re Application of:**<br><br>John H. Crowe, et al.<br><br>**Application No.:** Unknown<br><br>**Filing Date:**<br><br>**Title:** BIOLOGICAL SAMPLES AND METHOD FOR STABILIZING BIOLOGICAL SAMPLES | **D cket No.:** 010023-001200US<br><br>**Re:** Filing Patent Application |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**EXPRESS MAIL CERTIFICATE**

Utility Transmittal Letter;
Fee Transmittal Sheet;
Unsigned Declaration
Specification, Claims, Abstract and Drawings

I hereby certify that this paper and the enclosures listed above are being deposited with the U.S. Postal Service "Express Mail to Addressee" Express Mail No.: EU121945984US on October 15, 2003, and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

EU 121945984 US

Dated: 10/15/03

Respectfully submitted,

The Regents of the University of California

By: _____
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USA

UC Davis Case No. 2003-436-1

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**APPLICATION FOR PATENT**

# BIOLOGICAL SAMPLES AND METHOD FOR STABILIZING BIOLOGICAL SAMPLES

Inventors: John H. Crowe
Fern Tablin
Kamran Jamil
Ann E. Oliver
Xioacui Ma
James S. Clegg
Thomas MacRae

## Related Patent Applications

This patent application is related to co-pending patent application Serial No. 10/052,162, filed January 16, 2002. Patent application Serial No. 10/052,162 is a continuation-in-part patent application of co-pending patent application Serial No. 09/927,760, filed August 9, 2001. Patent application Serial No. 09/927,760 is a continuation-in-part patent application of co-pending patent application Serial No. 09/828,627, filed April 5, 2001. Patent application Serial No. 09/828,627 is a

continuation patent application of patent application Serial No. 09/501,773, filed February 10, 2000. All of the foregoing patent applications are fully incorporated herein by reference thereto as if repeated verbatim immediately hereinafter.

## Field of the Invention

Embodiments of the present invention generally broadly relate to biological samples, such as mammalian (e.g., human) cells, platelets, and the like. More specifically, embodiments of the present invention generally provide for the preservation and survival of biological samples.

Embodiments of the present invention also generally broadly relate to the therapeutic uses of biological samples; more particularly to manipulations or modifications of biological samples, such as loading biological samples with solutes (e.g., carbohydrates, such as trehalose) and preparing dried compositions that can be re-hydrated at the time of application. When biological samples for various embodiments of the present invention are re-hydrated, they are generally immediately restored to viability.

The compositions and methods for embodiments of the present invention are useful in many applications, such as in medicine, pharmaceuticals, biotechnology, and agriculture, and including transfusion therapy, as hemostasis aids and for drug delivery.

## Statement Regarding Federal Sponsored Research and Development

Embodiments of this invention were made with Government support under Grant Nos. N66001-00-C-8048 and N66001-02-C-8055, awarded by the Department of Defense Advanced Research Projects Agency (DARPA). Further embodiments of this invention were made with Government

support under Grant Nos. HL57810 and HL61204, awarded by the National Institutes of Health. The Government has certain rights to embodiments of this invention.

## Background of the Invention

A biological sample includes cells and blood platelets. A cell is typically broadly regarded in the art as a small, typically microscopic, mass of protoplasm bounded externally by a semi-permeable membrane, usually including one or more nuclei and various other organelles with their products. A cell is capable either alone or interacting with other cells of performing all the fundamental function(s) of life, and forming the smallest structural unit of living matter capable of functioning independently.

Cells may be transported and transplanted; however, this requires cryopreservation which includes freezing and subsequent reconstitution (e.g., thawing, re-hydration, etc.) after transportation. Unfortunately, a very low percentage of cells retain their functionality after undergoing freezing and thawing. While some cryoprotectants, such as dimethylsulfoxide, tend to lessen the damage to cells, they still do not prevent some loss of cell functionality.

Blood platelets are typically generally oval to spherical in shape and have a diameter of 2-4 μm. Today

platelet rich plasma concentrates are stored in blood bags at 22° C; however, the shelf life under these conditions is limited to five days. The rapid loss of platelet function during storage and risk of bacterial contamination complicates distribution and availability of platelet concentrates. Platelets tend to become activated at low temperatures. When activated they are substantially useless for an application such as transfusion therapy. Therefore, the development of preservation methods that will increase platelet lifespan is desirable.

Trehalose has been found to be suitable in the preservation of cells and platelets. Trehalose is a disaccharide found at high concentrations in a wide variety of organisms that are capable of surviving almost complete dehydration. Trehalose has been shown to stabilize membranes, proteins, and certain cells and platelets during drying (e.g., freeze-drying) in vitro.

Spargo et al., U.S. Patent No. 5,736,313, issued April 7, 1998, have described a method in which platelets are loaded overnight with an agent, preferably glucose, and subsequently lyophilized. The platelets are preincubated in a preincubation buffer and then are loaded with carbohydrate, preferably glucose, having a concentration in the range of about 100 mM to about 1.5 M. The incubation is taught to be conducted at about 10°C to about 37°C, most preferably about 25° C.

U.S. Patent No. 5,827,741, Beattie et al., issued October 27, 1998, discloses cryoprotectants for human cells and platelets, such as dimethylsulfoxide and trehalose. The cells or platelets may be suspended, for example, in a solution containing a cryoprotectant at a temperature of about 22°C and then cooled to below 15°C. This incorporates some cryoprotectant into the cells or platelets, but not enough to prevent hemolysis of a large percentage of the cells or platlets.

Accordingly, a need exists for the effective and efficient preservation of biological samples, such as platelets and cells, and the like. More specifically, and accordingly further, a need also exists for the effective and efficient preservation of platelets and cells (e.g., erythrocytic cells, eukaryotic cells, or any other cells, and the like), such that the preserved platelets and cells respectively maintain their biological properties and may readily become viable after storage.

## Summary of Embodiments of the Invention

In one embodiment of the present invention, a generally dehydrated composition is provided to comprise dried biological samples selected from a mammalian species (e.g., a human) and having a stress protein and being effectively loaded internally (e.g., uptaking external carbohydrate via fluid phase endocytosis) with at least about 10 mM of a carbohydrate (e.g., an oligosaccharide, such as trehalose) therein to preserve biological

properties during drying (e.g., air drying, vacuum drying, freeze drying, or etc.) and re-hydration. The amount of the carbohydrate inside the dried biological samples is preferably the amount obtained from maintaining a positive loading gradient or loading efficiency gradient on the biological samples. When the carbohydrate is trehalose, the amount of trehalose loaded inside the dried biological samples is preferably from about 10 mM to about 60 mM. In an embodiment of the invention, the dehydrated mammalian biological samples comprise at least one protein having at least about 0.05 % by weight of the stress protein, and at least about 5 mM of the carbohydrate. The dehydrated mammalian biological samples may additionally comprise at least about 0.01 gram water per gram of dry weight of biological sample.

Embodiments of the present invention further provide a process of preparing loaded biological samples comprising providing biological samples (preferably selected from a mammalian species, such as a human) having a stress protein; and loading (e.g., preferably with an oligosaccharide solution and/or with or without a fixative) an oligosaccharide (e.g., trehalose) into the biological samples at a temperature greater than about 25°C (e.g., greater than about 25°C but less than about 50°C, such as from about 30°C to less than about 50°C, or from about 30°C to about 40°C) to produce loaded biological samples. The loading preferably comprises taking up external oligosaccharide via fluid phase endocytosis from an oligosaccharide solution at the temperature greater than about 25°C. The loading further comprises incubating the biological samples at the temperature greater than about

25°C with the oligosaccharide solution. For these and various other embodiments of the present invention, the biological samples are preferably human biological samples, such as, by way of example only, blood platelets, erythrocytic cells (i.e., red blood cells), and eukaryotic cells selected from the group of eukaryotic cells consisting of mesenchymal stem cells and epithelial 293H cells. A biological sample produced in accordance with the foregoing method is provided.

In other embodiments of the present invention, a method is provided for loading (e.g., by fluid phase endocytosis) a solute and a stress protein into biological samples (e.g., an erythrocytic cell). Embodiments of the invention include loading the biological samples with a stress protein, such as by transfecting or loading by uptaking a stress protein from a solution containing the stress protein. The stress protein-loaded biological samples are then disposed in a solution having a solute (e.g., an oligosaccharide, such as trehalose) concentration of sufficient magnitude such that the solute is transferred from the solution into the biological samples. The stress protein-loaded biological samples are typically dispersed within the solution having the solute. The transferring of the solute from the solution is preferably by fluid phase endocytosis and preferably without degradation of the solute.

The method for various embodiments of the invention may additionally comprise preventing a decrease in a loading efficiency gradient in the loading of the solute

into the biological samples. In an embodiment of the invention where the solute comprises an oligosaccharide, the preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the biological samples may comprise maintaining a concentration of the oligosaccharide in the oligosaccharide solution below a certain concentration, such as below from about 35 mM to about 65 mM, more particularly below a concentration ranging from about 40 mM to about 60 mM, more particularly further below a concentration ranging from about 45 mM to about 55 mM (e.g., below about 50 mM). In another embodiment of the invention, the preventing a decrease in a loading efficiency gradient in the loading of the oligosaccharide into the biological samples comprises maintaining a positive gradient of loading efficiency to concentration of the oligosaccharide in the oligosaccharide solution.

In other embodiments of the present invention, a method is provided for loading (e.g., by fluid phase endocytosis) a solute and a stress protein into biological sample(s) (e.g., an erythrocytic cell). The biological sample(s) are disposed in a solution having a solute (e.g., an oligosaccharide, such as trehalose) and a stress protein such that the solute and the stress protein are transferred from the solution into the biological sample(s). The transferring of the solute and the biological samples is preferably by fluid phase endocytosis and preferably without degradation of the solute and/or the stress protein. The biological sample(s) containing the solute and the stress protein may then be dried and stored.

Embodiments of the present invention also provide a method for increasing the survival of a biological sample (e.g., a mammalian biological sample) comprising providing a biological sample having a stress protein (e.g., p26), loading the biological sample with a carbohydrate to produce a loaded biological sample, and dehydrating the loaded biological sample while maintaining a residual water content in the biological sample of at least about 0.01 g water per gram of dry weight of biological sample to increase survival of the biological sample. The method for increasing the survival of a biological sample may additionally comprise storing the dehydrated loaded biological sample to produce a stored biological sample, and rehydrating the stored biological sample. Providing a biological sample having a stress protein may comprise transfecting the biological sample with the stress protein, or loading the biological sample with the stress protein from a solution having the stress protein. Loading of the biological sample with a carbohydrate may comprise disposing a biological sample in a solution having the carbohydrate and a temperature of at least $25^0$ C for transferring (e.g., by fluid phase endocytosis) the carbohydrate from the solution into the biological sample.

Embodiments of the present invention further also provide a method for proliferating biological samples comprising providing biological samples having a stress protein, dehydrating the biological samples, rehydrating the biological samples to produce rehydrated biological samples, and proliferating the rehydrated biological samples. Dehydrating the biological samples may include maintaining a residual water content in the biological

sample of at least about 0.01 gram water per gram of dry weight of biological samples to increase survival of the biological samples. The method for proliferating biological samples may additionally comprise storing, prior to rehydrating the biological samples, the dehydrated biological samples.

Embodiments of the invention further provide a method for preparing a biological sample for treatment comprising providing a biological sample, and inserting into the biological sample cDNA for p26 cloned from *Artemia franciscana* so the biological sample produces p26. The biological sample may comprise a eukaryotic cell. The embodiments of the invention also provide a biological sample produced in accordance with the foregoing method.

These provisions, together with the various ancillary provisions and features which will become apparent to those skilled in the art as the following description proceeds, are attained by the processes and cells of the present invention, preferred embodiments thereof being shown with reference to the accompanying drawings, by way of example only, wherein:

## Brief Description of the Drawings

Figure 1 is a graph of viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with both the transfected 293 cells and the control 293 cells having no trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having no trehalose.

Figure 2 is a graph of viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with the transfected 293 cells and the control 293 cells both having no trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having 150 mM trehalose.

Figure 3 is a graph of viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with both the transfected 293 cells and control 293 cells having trehalose internally and with the drying buffer for both the transfected 293 cells and control 293 cells having 150 mM trehalose.

Figure 4 is a graph of the number of colonies formed after rehydration vs water content after drying the transfected 293 cells (T-293 cells) and the control 293 cells (293-cells)to 0.3 gm. water/gm. dry weight and to 0.2 gm. water/gm. dry weight, with both the transfected 293

cells and the control 293 cells having trehalose internally.

## Detailed Description of Preferred Embodiments of the Invention

Embodiments of the present invention broadly include biological samples, preferably mammalian (e.g., human) biological samples. Embodiments of the present invention further broadly include methods for preserving biological samples, as well as biological samples that have been manipulated (e.g., by drying to produce dehydrated biological samples) or modified (e.g., loaded with a chemical or drug) in accordance with methods of the present invention. Embodiments of the present invention also further broadly include methods for increasing the survival of biological samples, especially during drying and/or following drying, storing and rehydrating.

Biological samples for various embodiments of the present invention comprise any suitable biological sample, such as blood platelets and cells. The cells may be any type of cell including, not by way of limitation, erythrocytic cells, eukaryotic cells or any other cell, whether nucleated or non-nucleated.

The term "erythrocytic cell" is used to mean any red blood cell. Mammalian, particularly human, erythrocytes are preferred. Suitable mammalian species for providing erythrocytic cells include by way of example only, not only

human, but also equine, canine, feline, or endangered species.

The term "eukaryotic cell" is used to mean any nucleated cell, i.e., a cell that possesses a nucleus surrounded by a nuclear membrane, as well as any cell that is derived by terminal differentiation from a nucleated cell, even though the derived cell is not nucleated. Examples of the latter are terminally differentiated human red blood cells. Mammalian, and particularly human, eukaryotes are preferred. Suitable mammalian species include by way of example only, not only human, but also equine, canine, feline, or endangered species.

The source of the eukaryotic cells may be any suitable source such that the eukaryotic cells may be cultivated in accordance with well known procedures, such as incubating the eukaryotic cells with a suitable serum (e.g., fetal bovine serum). After the eukaryotic cells are cultured, they are subsequently harvested by any conventional procedure, such as by trypsinization, in order to be loaded with a protective preservative. The eukaryotic cells are preferably loaded by growing the eukaryotic cells in a liquid tissue culture medium. The preservative (e.g., an oligosaccharide, such as trehalose) is preferably dissolved in the liquid tissue culture medium, which includes any liquid solution capable of preserving living cells and tissue. Many types of mammalian tissue culture media are known in the literature and available from commercial suppliers, such as Sigma Chemical Company, St. Louis, Mo.,

USA: Aldrich Chemical Company, Inc., Milwaukee, Wis., USA; and Gibco BRL Life Technologies, Inc., Grand Island, N.Y., USA. Examples of media that are commercially available are Basal Medium Eagle, CRCM-30 Medium, CMRL Medium-1066, Dulbecco's Modified Eagle's Medium, Fischer's Medium, Glasgow Minimum Essential Medium, Ham's F-10 Medium, Ham's F-12 Medium, High Cell Density Medium, Iscove's Modified Dulbecco's Medium, Leibovitz's L-15 Medium, McCoy's 5A Medium (modified), Medium 199, Minimum Essential Medium Eagle, Alpha Minimum Essential Medium, Earle's Minimum Essential Medium, Medium NCTC 109, Medium NCTC 135, RPMMI-1640 Medium, William's Medium E, Waymouth's MB 752/1 Medium, and Waymouth's MB 705/1 Medium.

For various embodiments of the present invention, a biological sample is provided comprising at least one protein having at least about 0.05 % by weight of a stress protein, preferably at least about 0.07 % by weight of a stress protein, more preferably at least about 0.10 % by weight of a stress protein. The % by weight of the stress protein is the % by weight of the stress protein in the at least one protein (e.g., % by weight of the total non-yolk protein in the biological sample). Preferably, the protein (e.g., total protein) in the biological sample comprises from about 0.05 % by weight to about 20.0 % by weight of a stress protein, more preferably from about 0.07 % by weight to about 13.0 % by weight of a stress protein, most preferably from about 0.10 % by weight to about 1.00 % by weight (e.g. from about 0.10 % by weight to about 0.15 % by weight) of a stress protein. The concentration or weight % of the stress protein in the biological sample(s) may be

measured by any suitable means, such as with the Pierce BCA™ protein kit with BSA as standard.

The biological sample also comprises at least about 5 mM of a solute, preferably at least about 10 mM of a solute, more preferably at least about 20 mM of a solute. The amount of solute in the biological sample is the concentration (mM) of the solute in the biological sample, preferably in the cytosol of the biological sample before drying when the biological sample comprises cells. Preferably, the biological sample comprises from about 5 mM to about 65 mM of a solute, more preferably from about 10 mM to about 60 mM of a solute, most preferably from about 20 mM to about 40 mM (e.g. from about 20 mM to about 30 mM) of a solute. The solute preferably comprises a carbohydrate.

For various additional embodiments of the present invention, a biological sample (e.g., particularly a dried biological sample) is provided as also comprising at least about 0.01 g $H_2O$/g of dry weight of biological sample, preferably at least about 0.10 g $H_2O$/g of dry weight of biological sample, more preferably at least about 0.20 g $H_2O$/g of dry weight of biological sample. The g $H_2O$/g of dry weight biological sample is preferably the g $H_2O$/g of dry weight of biological sample after drying or dehydration. Preferably, the biological sample comprises from about 0.01 g $H_2O$/g of dry weight of biological sample to about 3.0 g $H_2O$/g of dry weight of biological sample (e.g., from about 0.01 to about 2.0 g $H_2O$/g of dry weight of biological

sample), more preferably from about 0.10 g $H_2O$/g of dry weight of biological sample to about 1.0 g $H_2O$/g of dry weight of biological sample (e.g., from about 0.1 to about 0.5 g $H_2O$/g of dry weight of biological sample), most preferably from about 0.20 g $H_2O$/g of dry weight of biological sample to about 0.5 g $H_2O$/g of dry weight of biological sample (e.g. from about 0.20 to about 0.30 g $H_2O$/g of dry weight of biological sample).

The carbohydrate (e.g., an oligosaccharide) may be a carbohydrate selected from the following groups of carbohydrates: a monosaccharide, an oligosaccharide (e.g., bioses, trioses, tetroses, pentoses, hexoses, heptoses, etc), a disaccharide (e.g., lactose, maltose, sucrose, melibiose, trehalose, etc), a trisaccharide (e.g., raffinose, melezitose, etc), or tetrasaccharides (e.g., lupeose, stachyose, etc), and a polysaccharide (e.g., dextrins, starch groups, cellulose groups, etc). More preferably, the carbohydrate is a disaccharide, with trehalose being the preferred, particularly since it has been discovered that trehalose does not degrade or reduce in complexity upon being loaded. Thus, in the practice of various embodiments of the invention, trehalose is transferred from a solution into the biological sample without degradation of the trehalose.

Stress proteins for embodiments of the present invention are also known as heat shock proteins (HSP) or molecular chaperones. Stress proteins assist the folding of proteins, reduce stress-associated protein denaturation and aggregation, aid in renaturation, and influence the final

intracellular location of mature proteins. Stress proteins are divided into groups or families, including Hsp100, Hsp90, Hsp70, Hsp60 (the chaperonins), and the small heat shock/ α (alpha) crystallin proteins, sometimes referred to as α-Hsps. Small heat shock proteins including α (alpha) crystallin proteins are low molecular weight heat shock proteins, ranging in size from about 10- to 40-kDa monomer molecular mass, but oli-gomerize into particles of varying monomer numbers. The functions of chaperones differ, but their activities are interrelated and often dependent on association into macromolecular complexes, sometimes consisting of representatives from more than one group or family.

P26 belongs to the α crystallin or α-Hsps group or family. As with many other Hsps, α-Hsps or α crystallin proteins protect cells during stress by preventing aggregation of unfolded proteins and in some cases assisting in their renaturation. As indicated, p26 is a small heat shock/ α crystallin protein, and has a diameter of about 15 nm, or about 520 kDa. It has 28 subunits, each being about 20.7 kDa in diameter. When biological samples are nucleated cells, stress causes p26 to move into the nucleus of the nucleated cell.

P26 is only found in the encysted embryos of the primitive, crustacean *Artemia franciscana* (i.e., shrimp embryos). Encysted embryos of the crustacean *Artemia franciscana* contain very large amount of the α-Hsp or p26, making up from about 12 % to about 15 % by weight of the total nonyolk protein. The remarkable stress resistance of *Artemia* cysts including p26 protects shrimp embryo cells

during encystment, diapause, and anaerobic quiescence, and prevents the aggregation of other proteins when shrimp embryos experience stresses of various kinds; thus playing an important role in their growth and development. For a comprehensive discussion of p26, including the procedure on how to purify p26 to homogeneity and how to measure the concentration of p26 (e.g., 28-mer), see *Influence of trehalose on the molecular chaperone activity of p26, a small heat shock/ α-crystallin protein* by Rosa I. Viner and James S. Clegg., Cell Stress Society International, Cell Stress & Chaperones (6 (2), pp. 126-135, 2001), fully incorporated herein by reference thereto as if repeated verbatim immediately hereinafter. For another comprehensive discussion of p26, including the cloning and sequencing a cDNA for p26, the listing of the complete sequence of p26-3-6-3 and the deduced amino acid sequence of p26, and the comparison of the deduced amino acid sequence of p26 to other small heat shock/ α-crystallin proteins (e.g., αA-crystallin, human αB-crystallin, human small heat shock protein 27 (H27), and a *Drosophila* small heat shock protein known as embryonal lethal (2) 13-1 (*Dro*)), see *Molecular Characterization of a Small Heat Shock/alpha-Crystallin Protein in Encysted Artemia Embryos* by Ping Liang, Reinout Amons, James S. Clegg, and Thomas H. MacRae, The Journal of Biological Chemistry (Vol. 272, No. 30, pp. 19051-19058, July 25, 1997), fully incorporated herein by reference thereto as if repeated verbatim immediately hereinafter. P26 may be readily purchased commercially.

The biological sample(s) may be provided with or loaded with or infused with the stress protein at any suitable time and by any suitable manner. More

specifically, the biological sample(s) may be provided, loaded, or infused, with the stress protein before loading with the solute, simultaneously with the loading of the solute, or after the loading of the solute.

In an embodiment of the present invention, the biological sample(s), particularly for nucleated cells, may be transfected with a DNA (e.g., cDNA) that codes for a particular stress protein so that the biological sample(s) produce its/their own stress protein. It is well known that transfecting comprises treating a biological sample with a plasmid (e.g., viral DNA) preferably having a viral promoter. Thus, the viral DNA or plasmid is the vehicle or device that can carry a cDNA of choice into a biological sample of choice. It is also well known that a catalytic agent, such as a catalytic element or compound ( e.g., Lipofectamine, which is commercially available), may be used to assist the plasmid and the viral promoter to enter the biological sample(s). Once inside the biological sample(s), the viral promoter, as well as the particular cDNA of choice that codes for the stress protein, enters the cellular DNA of the biological sample(s). The viral promoter may subsequently activate or "turn on" the cDNA of choice. The biological sample(s), particularly nucleated cells, subsequently produce the protein of interest within their own cellular machinery.

In an embodiment of the invention where the stress protein comprises p26, the biological sample(s), particularly nucleated cells, may be transfected by treating the biological sample(s) with the plasmid preferably comprising plasmid pSecTag2A, which may be

purchased commercially from Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California, 92008, USA. The plasmid includes cDNA (e.g., a stoichiometric quantity of cDNA) for p26 cloned from *Artemia franciscana*. cDNA may be prepared by any suitable procedure, such as the procedure described in the published literature entitled *Molecular Characterization of a Small Heat Shock/alpha-Crystallin Protein in Encysted Artemia Embryos* by Ping Liang, Reinout Amons, James S. Clegg, and Thomas H. MacRae, The Journal of Biological Chemistry (Vol. 272, No. 30, pp. 19051-19058, July 25, 1997), fully incorporated herein by reference thereto as if repeated verbatim immediately hereinafter. cDNA codes for p26 in the biological sample(s) to cause the development of p26 within the biological sample(s). The plasmid also preferably includes the viral promoter(e.g., a stoichiometric quantity of the viral promoter), CMV (cytomegalovirus) promoter which has been inserted into or combined with the pSecTag2A plasmid by the commercial supplier, such the Invitrogen Corporation. CMV refers to a DNA sequence in the transfection vector (pSecTag2) and is the human cytomegalovirus immediate-early promoter/enhancer. CMV permits efficient, high-level expression of the recombinant protein in transfected cells. The CMV promoter activates cDNA once inside the biological sample(s) of choice. The catalytic agent or compound sold by Invitrogen Corporation under the product name Lipofectamine 2000 may be used to assist the plasmid and cDNA to enter the biological sample(s).

In an embodiment of the invention, the p26 gene (p26 cDNA) may be ligated into the viral DNA (pSecTag2A DNA) by use of T4 DNA ligase and then cloned in *Escherichia coli*

DH5α. The Lipofectamine 2000 and the p26-containing plasmid (viral DNA) produced by ligation may be mixed in a tube and incubated at room temperature for a suitable period of time (e.g., 5 to 30 minutes) before application to the biological sample(s) for transfection. Thus, and as indicated, the p26 cDNA, the plasmid (viral DNA) and the viral promoter may be combined (e.g., stoichiometrically combined) into a single plasmid mixture (i.e., a plasmid DNA mixture), which may then be admixed with Lipofectamine 2000 and with a suitable inert solution, such as serum-free DMEM, to produce a transfecting solution. DMEM, Dulbecco's Modified Eagle Medium, is the medium in which the biological sample(s) (e.g., cells) may be grown. DMEM may be purchased commercially from Invitogen Corporation.

For various embodiments of the invention, the transfecting solution comprises from about 0.0001 weight % to about 0.005 weight % of the plasmid DNA mixture, from about 1.00 weight % to about 10.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 70.00 weight % to about 99.00 weight % of the inert solution (e.g., serum-free DMEM); more preferably from about 0.0002 weight % to about 0.002 weight % of the plasmid DNA mixture, from about 2.00 weight % to about 8.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 90.00 weight % to about 98.00 weight % of the inert solution (e.g., serum-free DMEM); and most preferably from about 0.0005 weight % to about 0.0015 weight % of the plasmid DNA mixture, from about 3.00 weight % to about 5.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 93.00 weight % to about 97.00 weight % of the inert solution (e.g., serum-

free DMEM). For transfecting, the biological sample(s) may be treated with the transfecting solution in any suitable manner, such as by immersing the biological sample(s) in the transfecting solution for a suitable period of time (e.g., 10 to 50 minutes). In an embodiment of the invention, transfections of biological sample(s) was accomplished by the use of 800 nanograms of the plasmid DNA mixture and 3 µl of Lipofectamine 2000 in 60 µl of serum-free DMEM for biological sample(s) in each well of a six-well culture plate. It is to be understood that the biological sample(s) may be transfected with the stress protein before being loaded with the solute, after being loaded with the solute, or simultaneously with the loading of the solute.

In another embodiment of the invention, the stress protein may be loaded into the biological sample(s) (i.e., into non-transfected biological sample(s)) by any suitable means and/or method(s), such as by the employment of a protein-loading solution (e.g., a p26-loading solution). For this embodiment of the invention, the stress protein, preferably the stress protein in essentially pure form, would be mixed with a suitable protein-loading solution (e.g., a p26-loading solution), and the biological sample(s) would subsequently be disposed in the protein-loading solution for causing the transfer of the stress protein from the protein-loading solution into the biological sample(s). The protein-loading solution may be any suitable physiologically acceptable solution in an amount and under conditions effective to cause uptake or "introduction" of the stress protein from the protein-loading solution into the biological sample(s). Broadly, by

way of example only, a physiological acceptable solution comprises one or more of the following: the stress protein (e.g., p26), a salt solution (e.g., PBS), a protein (e.g., BSA), and a carbohydrate (e.g., a starch, an oligosaacharide, etc). In other embodiments of the invention, the physiological acceptable solution comprises one or more of the following: the plasmid DNA mixture (e.g., the plasmid DNA mixture), Lipofectamine 2000, a salt solution (e.g., PBS), a protein (e.g., BSA), and a carbohydrate (e.g., a starch, an oligosaacharide, etc).

More specifically, by way of example only, a physiological acceptable solution for various embodiments of the invention comprises one or more of the following: from about 0.0001 weight % to about 0.005 weight % of the plasmid DNA mixture, from about 1.00 weight % to about 10.00 weight % of the catalytic agent (e.g., Lipofectamine 2000), from about 50.00 weight % to about 99.00 weight % of the salt solution , from about 1.00 weight % to about 20.00 weight % of the protein, and from about 1.00 weight % to about 20.00 weight % of the carbohydrate; more preferably from about 0.0002 weight % to about 0.002 weight % of the plasmid DNA mixture, from about 2.00 weight % to about 8.00 weight % of the catalytic agent (e.g., Lipofectamine 2000), from about 70.00 weight % to about 98.00 weight % of the salt solution , from about 2.00 weight % to about 10.00 weight % of the protein, and from about 2.00 weight % to about 10.00 weight % of the carbohydrate; and most preferably from about 0.0005 weight % to about 0.0015 weight % of the plasmid DNA mixture, from about 3.00 weight % to about 5.00 weight % of the catalytic agent (e.g., Lipofectamine 2000), from about 80.00 weight % to

about 95.00 weight % of the salt solution , from about 3.00 weight % to about 8.00 weight % of the protein, and from about 3.00 weight % to about 5.00 weight % of the carbohydrate.

More specifically further, by way of example only, a physiological acceptable solution for other various embodiments of the invention comprises one or more of the following: from about 1.00 weight % to about 20.00 weight % of the stress protein (e.g., p26), from about 50.00 weight % to about 99.00 weight % of the salt solution, from about 1.00 weight % to about 20.00 weight % of the protein, and from about 1.00 weight % to about 20.00 weight % of the carbohydrate; more preferably from about 2.00 weight % to about 15.00 weight % of the stress protein, from about 70.00 weight % to about 98.00 weight % of the salt solution, from about 2.00 weight % to about 10.00 weight % of the protein, and from about 2.00 weight % to about 10.00 weight % of the carbohydrate; and most preferably from about 3.00 weight % to about 10.00 weight % of the stress protein, from about 80.00 weight % to about 95.00 weight % of the salt solution , from about 3.00 weight % to about 8.00 weight % of the protein, and from about 3.00 weight % to about 5.00 weight % of the carbohydrate.

The loading temperature of the protein-loading solution for loading a stress protein into the biological sample(s) may be any suitable temperature, such as a temperature ranging from about 0 degrees C to about 60 degrees C, preferably from about 10 degrees C to about 40 degrees C, more preferably from about 36 degrees C to about 38 degrees C. The loading/incubating time for loading the

stress protein may be any suitable time, such as a time ranging from about 10 minutes to about 46 hours, more preferably from about 30 minutes to about 34 hours, most preferably from about 45 minutes to about 24 hours.

In another embodiment of the invention the stress protein may be delivered into the biological sample(s) through the use of a drug delivery kit sold under the trademark BioPORTER to the Sigma-Aldrich Company. Suitable BioPORTER brand drug delivery kits are sold under product Nos. BPQ24 and BPQ96. The BioPORTER brand drug delivery kit has a BioPORTER reagent which reacts quickly and interacts non-covalently with the stress protein (e.g., p26) for creating a protective vehicle for immediate delivery into biological sample(s). In embodiments of the invention the BioPORTER reagent is incubated with the stress protein (e.g., p26) for a suitable period of time, such as from about 2 mins. to about 15 mins. (e.g., 5 mins.). Subsequently, the resulting incubated product having the stress protein is then incubated with the biological sample(s) for 1 to 8 hours (e.g., about 4 hours). In an embodiment of the invention, the bioporter reagent-stress protein complex is taken up by fluid phase endocytosis, subsequently fusing with a membrane (e.g., an endosome membrane) of the biological sample(s) and releasing the stress protein into the biological sample(s)(e.g., into the cytosol of the biological sample(s)). The foregoing procedure may also be employed for loading the solute simultaneously with the loading of the stress protein.

Embodiments of the present invention will be explained by loading of the solute into the biological sample(s)

after the biological sample(s) contain the stress protein. However, it is to be understood that the spirit and scope of the present invention includes loading or transfecting the biological sample(s) with the stress protein after the biological sample(s) is/are loaded with the solute, or simultaneously with the loading of the solute. Thus, embodiments of the invention are not to be restricted to any particular order with respect to loading, or transfecting, the biological sample(s) with the stress protein, and the loading of the solute into the biological sample(s). The loading, or transfecting, the biological sample(s) with the stress protein may be: (i)before the biological sample(s) is/are loaded with the solute; (ii)after the biological sample(s) has/have been loaded with the solute; or (iii) simultaneously with the loading of the solute.

After the biological sample(s) has/have been transfected with/by, or has/have been loaded with, a desired amount of the stress protein (e.g., p26), the biological sample(s) may then be loaded with a suitable solute. Broadly, the preparation of solute-loaded biological sample(s) containing the stress protein in accordance with embodiments of the invention comprises the steps of loading one or more biological sample(s) with a solute by placing one or more biological sample(s) in a solute solution having a solute concentration of sufficient magnitude for transferring the solute from the solution into the biological sample(s). For increasing the transfer or uptake of the solute from the solute solution, the solute solution temperature or incubation temperature has a

temperature above about 25°C, more preferably above $30^0$ C, such as from about $30^0$ C to about $40^0$ C.

The method(s) for various embodiments of the present invention may additionally comprise preventing a decrease in a loading gradient and/or a loading efficiency gradient in the loading of the solute into the biological sample(s). Preventing a decrease in a loading efficiency gradient in the loading of the solute into the biological sample(s) comprises maintaining a positive gradient of loading efficiency (e.g., in %) to concentration (e.g., in mM) of the solute in the solute solution. Preventing a decrease in a loading gradient in the loading of the solute into the biological sample(s) comprises maintaining a concentration of the solute in the solute solution below a certain concentration (e.g., below a concentration ranging from about 35 mM to about 65 mM, more particularly below from about 40 mM to about 60 mM, or below from about 45 mM to about 55 mM, such as below about 50 mM); and/or maintaining a positive gradient of concentration of solute loaded into the biological sample(s) to concentration of the solute in the solute solution. It is to be understood that the foregoing embodiments of the invention would apply to biological sample(s): (i)before being loaded with, or transfected by, a stress protein; (ii)after having been loaded with, or transfected by, a stress protein; or (iii) simultaneously with the loading or transfecting of the biological sample(s) with the stress protein.

The solute solution for various embodiments of the present invention may be used for loading and/or drying and/or rehydration, or for any other suitable purpose.

When the solute solution is employed for loading a solute into the biological sample(s), the solute solution may be any suitable physiologically acceptable solution in an amount and under conditions effective to cause uptake or "introduction" of the solute from the solute solution into the biological sample(s). A physiologically acceptable solution is a suitable solute-loading buffer, such as any of the buffers stated in the previously mentioned related patent applications, all having been incorporated herein by reference thereto. The solute solution may also be any suitable physiologically acceptable solution in an amount and under conditions effective for drying and/or rehydration. Therefore, the solute solution may be used as a drying buffer for drying loaded biological sample(s) and/or as a rehydration buffer for rehydrating biological sample(s) in reconstituting biological sample(s). Thus, any of the solute solutions for embodiments of the present invention may be used for any suitable purpose, including loading, drying, and rehydration.

For particular embodiments of the present invention, especially when the solute solution is being employed as a loading buffer, the solute solution comprises a solute (e.g., 50 mM to 150 mM trehalose) and a salt solution (e.g., a growth medium, such as PBS). In other particular embodiments of the invention, especially when the solute solution is being employed as a drying buffer and/or a rehydration buffer, the solute solution comprises one or more of the following: a salt solution (e.g., PBS), a protein, a solute and an acid (e.g., HEPES, or N-(2-hydroxyl ethyl) piperarine-N'-(2-ethanesulfonic acid)). However, it is to be understood that the solute solution

comprising one or more of a salt solution, a protein, a solute, and an acid may be used for any other suitable purpose.

The salt solution may be any suitable physiologically acceptable solution in an amount and under conditions effective to function as a carrier medium for a solvent, or for a mixture of a solvent, a protein and/or an acid. The salt solution may comprise KCl and NaCl, such as more particularly about 1 to 15 mM KCl and about 40 to 80 mM NaCl with pH 7.2. The salt solution may also comprise a phosphate buffered saline (PBS) solution comprising NaCl, $Na_2HPO_4$, and $KH_2PO_4$. A suitable PBS buffer comprises a buffer sold under the product name Dulbecco's PBS (DPBS, Gibco cat # 14190), or a buffer comprising 283 mOsm PBS buffer (51.3 mM NaCl, 1.87 mM $Na_2HPO_4$, 0.35 mM $KH_2PO_4$, pH 7.2).

The acid may be any suitable acid. Preferably, the acid comprises a sulfonic acid, such as, by way of example only, 5 to 20 mM HEPES ( N-(2-hydroxyl ethyl) piperarine-N'-(2-ethanesulfonic acid)).

The carbohydrate for various embodiments of the invention is preferably trehalose. The amount of the preferred trehalose loaded inside the biological sample(s) ranges from about 10 mM to about 60 mM (e.g., up to about 50 mM), and is achieved by incubating the biological sample(s) to preserve biological properties during drying with a trehalose solution, preferably a trehalose solution that has up to about 60 mM (e.g., up to about 50 mM) trehalose therein. Higher concentrations of trehalose

during incubation are not preferred, particularly since an embodiment of the invention includes preventing a decrease in a loading gradient, or a loading efficiency gradient, in the loading of the solute into the biological sample(s). It has been discovered that preventing a decrease in a loading gradient, or a loading efficiency gradient, in the loading of a solute (e.g., an oligosaccharide, such as the trehalose) into a biological sample(s) comprises maintaining a concentration of the solute in the solute solution below a certain concentration (e.g., below about 75 mM, such as below about a concentration ranging from about 35 mM to about 65 mM, more particularly below from about 40 mM to about 60 mM, or below from about 45 mM to about 55 mM, such as below about 50 mM). It has been further discovered that preventing a decrease in a loading gradient, or a loading efficiency gradient, in the loading of a solute into a biological sample(s) comprises maintaining a positive gradient of loading efficiency to concentration of the solute in the solute solution. The effective loading of trehalose is also preferably accomplished by means of using an elevated temperature of from greater than about 25° C to less than about 40° C, more preferably from about 30°C to less than about 40°C, most preferably about 37°C.

In an embodiment of the invention where the solute is to be loaded into the biological sample(s) simultaneously with the loading of the biological sample(s) with the stress protein, the solute solution comprises the stress protein, the solute, a salt solution (e.g., PBS) and optionally one or more of the following: a protein (e.g.,

BSA), and a carbohydrate (e.g., a starch). More specifically, the solute solution comprises from about 1.00 weight % to about 20.00 weight % of the stress protein (e.g., p26), from about 1.00 weight % to about 20.00 weight % of the solute, from about 50.00 weight % to about 99.00 weight % of the salt solution, and, optionally from about 1.00 weight % to about 20.00 weight % of the protein, and from about 1.00 weight % to about 20.00 weight % of the carbohydrate; more preferably from about 2.00 weight % to about 15.00 weight % of the stress protein, from about 2.00 weight % to about 10.00 weight % of the solute, from about 70.00 weight % to about 98.00 weight % of the salt solution , and, optionally, from about 2.00 weight % to about 10.00 weight % of the protein, and from about 2.00 weight % to about 10.00 weight % of the carbohydrate; and most preferably from about 3.00 weight % to about 10.00 weight % of the stress protein, from about 3.00 weight % to about 8.00 weight % of the solute, from about 80.00 weight % to about 95.00 weight % of the salt solution , and, optionally, from about 3.00 weight % to about 8.00 weight % of the protein, and from about 3.00 weight % to about 5.00 weight % of the carbohydrate. A commercial available loading media is sold under the product name DMEM (Gibco cat # 11965).

The loading temperature of the solute solution for this embodiment of the invention ranges from about 0 degrees C to about 60 degrees C, preferably from about 10 degrees C to about 40 degrees C, more preferably from about 36 degrees C to about 38 degrees C. The loading/incubating time for loading the stress protein may be any suitable time, such as a time ranging from about 10 minutes to about

46 hours, more preferably from about 30 minutes to about 34 hours, most preferably from about 45 minutes to about 24 hours.

In an embodiment of the invention where the solute is to be loaded into the biological sample(s) simultaneously with the transfecting of the biological sample(s) with the stress protein, the solute solution comprises the solute, the plasmid DNA mixture, the catalytic compound (e.g. Lipofectamine 2000), and the inert solution (e.g., serum-free DMEM). More specifically, the solute solution comprises from about 1.00 weight % to about 20.00 weight % of the solute, from about 0.0001 weight % to about 0.005 weight % of the plasmid DNA mixture, from about 1.00 weight % to about 10.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 70.00 weight % to about 99.00 weight % of the inert solution (e.g., serum-free DMEM); more preferably from about 2.00 weight % to about 10.00 weight % of the solute, from about 0.0002 weight % to about 0.002 weight % of the plasmid DNA mixture, from about 2.00 weight % to about 8.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 90.00 weight % to about 98.00 weight % of the inert solution (e.g., serum-free DMEM); and most preferably from about 3.00 weight % to about 8.00 weight % of the solute, from about 0.0005 weight % to about 0.0015 weight % of the plasmid DNA mixture, from about 3.00 weight % to about 5.00 weight % of the catalytic compound (e.g., Lipofectamine 2000), and from about 93.00 weight % to about 97.00 weight % of the inert solution (e.g., serum-free DMEM). The loading temperature of the solute solution for this embodiment of the invention ranges from about 0

degrees C to about 60 degrees C, preferably from about 10 degrees C to about 40 degrees C, more preferably from about 36 degrees C to about 38 degrees C. The loading/incubating time may be any suitable time, such as a time ranging from about 10 minutes to about 46 hours, more preferably from about 30 minutes to about 34 hours, most preferably from about 45 minutes to about 24 hours.

After the biological sample(s) have been effectively loaded with a solute, and preferably subsequently washed, the biological sample(s) may then be contacted with a drying buffer. The drying buffer preferably includes the solute, preferably in amounts up to about 3000 mM. The solute in the drying buffer assists in spatially separating the biological sample(s) as well as stabilizing the biological sample(s) membranes on the exterior. More specifically, the solute solution functioning as a drying buffer, may comprise at least about 50 mM of the solute, at least about 2.0 % by weight of the acid, at least about 0.5 % by weight of the protein, and at least about 25 mOsm for an osmolarity of the salt solution. More specifically further, the solute solution for drying buffer purposes may comprise the solute having a concentration ranging from about 50 mM to about 3000 mM, preferably from about 100 mM to about 1500 mM, more preferably from about 150 mM to about 1000 mM, most preferably from about 200 mM to about 600 mM. The osmolarity of the salt solution in the solute solution may range from about 25 mOsm to about 1000 mOsm, preferably from about 50 mOsm to about 300 mOsm, more preferably from about 75 mOsm to about 200 mOsm. The amount or quantity of the acid (e.g., HEPES) in the solute solution may range from about 2.0 % by weight to about 50 %

by weight, preferably from about 5 % by weight to about 35 % by weight, more preferably from about 10 % by weight to about 30 % by weight, most preferably from about 12 % by weight to about 20 % by weight (e.g., about 15 % by weight). The amount or quantity of the protein (e.g. HSA) in the solute solution may range from about 0.5 % by weight to about 15 % by weight, preferably from about 1 % by weight to about 10 % by weight, more preferably from about 1.5 % by weight to about 8 % by weight, most preferably from about 1.5 % by weight to about 5 % by weight (e.g., about 2.5 % by weight). The drying buffer may also include a bulking agent (to further separate the biological sample(s)). Albumin may serve as a bulking agent, but other polymers may be used with the same effect. If albumin is used, it is preferably from the same species as the biological sample(s). Suitable other polymers, for example, are water-soluble polymers such as HES (hydroxy ethyl starch) and dextran.

The solute-loaded, stress protein-contained biological sample(s) in the drying buffer may then be dried by any suitable means, such as by freeze drying, vacuum drying, or air drying, all well known in the art. If the solute-loaded, stress protein-contained biological sample(s) in the drying buffer are freeze-dried, the solute-loaded, stress protein-contained biological sample(s) in the drying buffer may be dried while simultaneously cooled to a temperature below about -32°C. A cooling, that is, freezing, rate is preferably between -30°C and -1°C/min. and more preferably between about -2°C/min to -5°C/min. Drying may be continued until about 95 weight percent of water has been removed from the biological sample(s).

During the initial stages of lyophilization, the pressure is preferably at about 10 x $10^{-6}$ torr. As the biological samples dry, the temperature can be raised to be warmer than -32°C. Based upon the bulk of the biological samples, the temperature and the pressure it can be empirically determined what the most efficient temperature values should be in order to maximize the evaporative water loss. Dried (e.g., freeze dried) biological sample compositions preferably have less than about 8 weight percent water.

The solute-loaded, stress protein-contained biological sample(s) in the drying buffer may be vacuum dried in accordance with well known procedures. Biological sample(s) loaded with trehalose and producing p26 may be aliquotted into volumes of 50-150 uL and subjected to vacuum in the range of 23 mm Hg at room temperature for a period in the range of 2 to 4 hours. This vacuum drying technique would bring the water content in the biological sample(s) down to about 0.2 gm. $H_2O$/gm. dry weight.

The solute-loaded, stress protein-contained biological sample(s) in the drying buffer may be air dried in accordance with well known procedures. Biological samples loaded with trehalose and producing p26 may be aliquotted into volumes of 50 uL - 1.0 mL and dried either in a biohood or in a desiccator modified to distribute a stream of dry air evenly across the surface of the biological sample(s). The drying may be conducted at room temperature for a period in the range of 6 to 10 hours. This air drying technique would bring the water content in the

biological sample(s) down to about 0.2 gm. $H_2O$/gm. dry weight.

Preferably, the biological samples are dried to a water content below about 20 gm. water/gm. dry weight biological sample(s), more preferably to a water content below about 15 gm. water/gm. dry weight biological sample(s), most preferably to a water content below about 8 gm. water/gm. dry weight biological sample(s) (e.g., below about 4 gm. water/gm. dry weight biological sample(s), such as to a water content ranging from about 0.01 gm. water/gm. dry weight biological sample(s) to about 4.0 gm. water/gm. dry weight biological sample(s). As previously indicated, a dried biological sample(s) may comprise at least about 0.01 g $H_2O$/g of dry weight of biological sample(s), preferably at least about 0.10 g $H_2O$/g of dry weight of biological sample(s), more preferably at least about 0.20 g $H_2O$/g of dry weight of biological sample(s). Preferably, the biological sample(s) comprises from about 0.01 g $H_2O$/g of dry weight of biological sample(s) to about 3.0 g $H_2O$/g of dry weight of biological sample(s) (e.g., from about 0.01 to about 2.0 g $H_2O$/g of dry weight of biological sample(s)), more preferably from about 0.10 g $H_2O$/g of dry weight of biological sample(s) to about 1.0 g $H_2O$/g of dry weight of biological sample(s) (e.g., from about 0.1 to about 0.5 g $H_2O$/g of dry weight of biological sample(s)), most preferably from about 0.20 g $H_2O$/g of dry weight of biological sample(s) to about 0.5 g $H_2O$/g of dry weight of biological sample(s) (e.g. from about 0.20 to about 0.30 g $H_2O$/g of dry weight of biological sample(s)).

It has been discovered that the biological sample(s) having a stress protein (e.g., p26) has/have a greater viability or survival after or following desiccation than control biological sample(s)(i.e., biological sample(s) not having a stress protein) have after desiccation. It has also been discovered that the biological sample(s) having a stress protein and a loaded solute (e.g., trehalose) has/have an even greater viability or survival after or following desiccation than control biological sample(s) not having a stress protein. The stress protein acts synergistically with the solute to stabilize the biological sample(s) and significantly improve the survival of biological sample(s) after desiccation. It has further also been discovered that the biological sample(s) having a stress protein, and no loaded solute, but dried in a drying buffer having the solute, has/have a greater viability or survival following desiccation than control biological sample(s) (i.e., biological sample(s) without a stress protein and dried in a drying buffer not having a solute) has/have after desiccation. Survival or viability of biological samples having a stress protein improves when dried in a drying buffer having a solute compared to biological samples dried in a drying buffer without a solute. The viability (e.g., the % viability) of biological sample(s) after drying may be determined by any suitable procedure well known to those skilled in the art, such as by way of example only, trypan blue exclusion. Trypan blue is a membrane-impermeant dye that, when exposed to the biological sample(s) for 3 min, will only stain biological sample(s) with compromised plasma membranes. The

biological sample(s) can be examined microscopically and counted. The % viability is calculated as the percentage of unstained (live) biological sample(s) out of the total number of biological sample(s).

The viability (e.g., the % viability) of biological sample(s) after drying may be determined by fluorescent live/dead analyses. There are several commercially available fluorescent live/dead kits. These kits work on the same principles as trypan blue; that is, dead biological sample(s) with compromised plasma membranes will take up membrane-impermeant dyes. A typical live/dead kit may contain a membrane permeant dye (e.g. syber green, SG, from Molecular Probes), which will stain all the biological sample(s), and a membrane-impermeant dye (e.g. propidium iodide, PI), which will stain only the dead biological sample(s). The percentage of dead biological sample(s) is calculated by counting the PI-stained biological sample(s) and dividing by the SG-stained biological sample(s). The percentage of viable biological sample(s) is calculated by subtracting the % dead biological sample(s) from 100.

After drying and storage of the biological sample(s), the process of using such a dehydrated biological-sample composition comprises rehydrating the biological sample. The rehydration preferably includes a prehydration step, sufficient to bring the water content of the dried biological sample(s) to between about 20 weight percent and about 50 percent, preferably from about 20 weight percent to about 40 weight percent. More preferably, when reconstitution of the dried biological sample(s) is desired, the dried biological sample(s) is/are prehydrated

in moisture saturated air at about 37°C for about one hour to about three hours, followed by rehydration. Use of prehydration yields biological sample(s) with a much more dense appearance and with no balloon biological sample(s) being present. The preferred prehydration step brings the water content of the dried biological sample(s) to between about 20 weight percent to about 50 weight percent. Rehydration or the prehydrated biological sample(s) may be with any aqueous based solutions, depending upon the intended application.

It has been discovered that the ability of dried biological sample(s) having a stress protein (e.g., p26) to proliferate and form colonies after rehydration is greater than the ability of dried biological sample(s) not having a stress protein to proliferate and form colonies. It has also been further discovered that the ability of dried biological sample(s) having a stress protein and a solute to proliferate and form colonies after rehydration is also greater than dried biological sample(s) not having a stress protein, or having a solute and no stress protein. The proliferation or the number of colonies formed by the biological sample(s) after drying and rehydration may be determined by any suitable procedure well known to those skilled in the art. By way of example only, after rehydration, the biological sample(s) may be plated into T-25 flasks and incubated at 37 °C for 7 days. The biological sample(s) may then be subsequently stained with either coomassie blue or Hema 3, and the colonies in each flask may be counted to obtain the proliferation or the

number of colonies formed by the biological sample(s) after drying and rehydration.

Embodiments (e.g., the viability of dried biological sample(s), proliferation of biological sample(s) after drying and rehydration, etc.) of the present invention will be illustrated with eukaryotic 293 cells (which are epithelial in origin) and by reference to Figures 1-4. It is to be understood that such use of 293 cells and such reference to Figures 1-4 are for exemplary purposes only, and are not to limit any of the embodiments of the present invention, or limit the spirit and scope of the present invention in general.

Referring in detail now to Figure 1, there is seen a graph of T-293 cell viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with both the transfected 293 cells and the control 293 cells having no trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having no trehalose. Example II below provides the more specific testing conditions and parameters which produced the graphical illustrations of Figure 1. Graph **10** and graph **12** in Figure 1 represents the transfected 293 cells, and the control 293 cells, respectively. Figure 1 illustrates that transfected T-293 cells transfected with p26 survive drying better than control 293 cells not having been transfected with p26. Alternatively, Figure 1 may illustrate that, when there is no trehalose inside or outside, there is no difference in survival between the two types of cells.

In Figure 2 there is seen a graph of viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with both the transfected 293 cells and the control 293 cells having no trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having 150 mM trehalose. Example III below provides the more specific testing conditions and parameters which produced the graphical illustrations of Figure 2. Graph **20** and graph **22** in Figure 2 represents the transfected 293 cells, and the control 293 cells, respectively. Figure 2 illustrates that survival of transfected T-293 cells is improved compared to control 293 cells when the transfected T-293 cells are dried in a drying buffer having 150 mM trehalose.

In Figure 3 there is seen a graph of viability (%) subsequent to drying vs. water content (gm. water/gm. dry weight) after drying for transfected 293 cells (T-293 cells) and for control 293 cells (293-cells), with both the transfected 293 cells and the control 293 cells having trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having 150 mM trehalose. Graph **30** and graph **32** in Figure 3 represents the transfected 293 cells, and the control 293 cells, respectively. Example IV below provides the more specific testing conditions and parameters which produced the graphical illustrations of Figure 3. Figure 3 illustrates that mammalian T-293 cells transfected with p26, and loaded with trehalose, and dried in a drying buffer having

trehalose greatly improves survival and/or viability when compared to control 293 cells not transfected with p26.

Figure 4 is a graph of the number of colonies formed after rehydration vs water content after drying the transfected 293 cells (T-293 cells) and the control 293 cells (293-cells)to 0.3 gm. water/gm. dry weight and to 0.2 gm. water/gm. dry weight, with both the transfected 293 cells and the control 293 cells having trehalose internally and with the drying buffer for both transfected 293 cells and control 293 cells having 150 mM trehalose. Blocks **40** and **44** respectively represent transfected 293 cells for water contents of 0.3 gm water/gm dry weight and to 0.2 gm water/gm dry weight. Block **42** and the number "0" represented by numeral **46** respectively represent control 293 cells for water contents of 0.3 gm water/gm dry weight and to 0.2 gm water/gm dry weight. Example V below provides the more specific testing conditions and parameters which produced the graphical illustrations of Figure 4. In the experiment that produced the results and graphical illustrations of Figure 4, transfected 293 cells and control cells were both dried respectively to 0.3 gm water/gm dry weight and to 0.2 gm water/gm dry weight, rehydrated and then plated (cultured) to determine their ability to form colonies subsequent to rehydration (a measure of long-term proliferation and survival). As illustrated in Figure 4, transfected T-293 cells dried to 0.3 gm water/gm dry weight were able to produce colonies 20X greater than the control 293 cells. This pattern persisted at lower water contents of 0.2 gm water/gm dry weight. However, no control 293 cells were able to proliferate at a water content of 0.2 gm water/gm dry

weight, while a significant fraction of the transfected T-293 cells did so.

Embodiments of the present invention will be illustrated by the following set forth examples which are being given to set forth the presently known best mode and by way of illustration only and not by way of any limitation. It is to be understood that all materials, chemical compositions and procedures referred to below, but not explained, are well documented in published literature and known to those artisans possessing skill in the art. All materials and chemical compositions whose source(s) are not stated below are readily available from commercial suppliers, who are also known to those artisans possessing skill in the art. All parameters such as concentrations, mixing proportions, temperatures, rates, compounds, etc., submitted in these examples are not to be construed to unduly limit the scope of the invention. Abbreviations used in the examples, and elsewhere, are as follows:

DMSO = dimethylsulfoxide

ADP = adenosine diphosphate

PGE1 = prostaglandin E1

HES = hydroxy ethyl starch

FTIR = Fourier transform infrared spectroscopy

EGTA = ethylene glycol-bis(2-aminoethyl ether) N,N,N',N', tetra-acetic acid

TES = N-tris (hydroxymethyl) methyl-2-aminoethane-sulfonic acid

HEPES = N-(2-hydroxyl ethyl) piperarine-N'-(2-ethanesulfonic acid)

PBS = phosphate buffered saline

HSA = human serum albumin

BSA = bovine serum albumin

ACD = citric acid, citrate, and dextrose

MβCD = methyl-β-cyclodextrin

## EXAMPLE I

p26 was purified from encysted embryos of *A franciscana* (San Francisco Bay) purchased from San Francisco Bay Brand, Newark, CA, USA. Purification steps were performedat $4^0$ C or on ice. Dried embryos (50 g) were hydrated at $4^0$ C for 16 hours in sea water; filtered; washed with cold 40 mM HEPES-KOH, pH 7.5, at $4^0$ C, 70 mM NaCl, and 1 mM EDTA (buffer A); and homogenized in the same buffer with a Retsch motorized mortar and pestle (Brinkman Instruments, Canada). The homogenate was centrifuged (4000 x *g*, 20 minutes) and the supernatant filtered through 6 layers of cheesecloth, centrifuged again at 16 000 x *g* for 40 minutes, and then at 23 500 x *g* for 30 minutes. Solid $(NH_4)_2SO_4$ was added to 40% saturation in the final supernatant. Precipitated proteins were collected at 10 000 x *g* for 30 minutes; dissolved in 20 mM Tris-HCl, pH 8.15, 150 mM NaCl, 1 mM $MgCl_2$, and 0.1 mM EDTA (buffer B); and dialyzed overnight against this buffer. After dialysis, the solution was passed through a 0.45-mm filter, applied to a Source 15 Q ion-exchange column (Amersham Pharmacia Biotech),

equilibrated, and developed at 2 mL/min in buffer B. The column was washed with buffer B for 30 minutes, and a linear NaCl gradient (150-500 mM) was used for elution of p26 between 235-270 mM NaCl. Fractions containing p26 were pooled; concentrated using Centriprep-30 (Amicon); dialyzed against 40 mM HEPES-KOH, pH 7.5 (buffer C), and 300 mM NaCl; further purified by gel filtration using a TSK-Gel G4000SW$_{XL}$ column (0.78 x 30 cm, Toso Haas, Japan); equilibrated; and developed at 0.5 mL/min in buffer C and 300 mM NaCl. p26 was eluted between 9.5-10.5 mL of the buffer volume, and the resulting protein was more than 95% pure. The protein was concentrated to approximately 1 mg/mL with Centriprep-30; dialyzed against 50 mM Tris-HCl, pH 8, and 2 mM EDTA (TE buffer); and centrifuged at 10 000 x *g* for 15 minutes. Further concentration and storage in buffer C led to unwanted insoluble aggregates. Aliquots were quick frozen in liquid nitrogen and stored at -70$^0$ C. Fractions from each step of purification were checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and/or Western immunoblotting with polyclonal antibody to p26 and then pooled according to purity.

## EXAMPLE II

293 cells and T293 cells were grown in T-25 flasks to ~90% confluence. The cells were harvested by trypsinization according to standard protocols. Briefly, the medium was removed from the cultures and they were washed one time with 5 mL DPBS. Trypsin (1 mL of 0.05% in0.53 mM EDTA-4Na) was added to the culture for ~1 min and

the flasks were rapped to dislodge the cells. Medium (4 mL) was added to stop the reaction, and the cells were pelleted by centrifugation at 176 x g for 5 min. The pellet was suspended in 5-10 mL DPBS and the centrifugation step was repeated. The cell pellet was then suspended in air drying buffer lacking trehalose (10 mM Hepes, 5 mM KCL, 65 mM NaCl, and 5.7% BSA with pH 7.2) at 1.4 million cells per mL. Aliquots (1.0 mL) were placed in 35 mm polysterene Petri dishes and air-dried in a ThermoForma biosafety cabinet in specific marked locations in the center of the hood over 0-24 hours. At various time points during drying, samples were removed for viability and water content analyses. Water contents were measured gravimetrically in triplicate. For viability measurements, samples were rehydrated with 1 mL medium. 50 µL of cellular suspension was mixed with 50 µL trypan blue and incubated at room temperature for 3 min. Cells were visualized at 10X by light microscopy on a hemacytometer and counted using five counts of 50-100 cells per 1 $mm^2$ hemocytometer grid square for each sample. Percent viability was calculated as the number of cells excluding the dye divided by the total number of cells. Viability was plotted as a function of water content as the means +/- standard deviation (for both variables), with the results illustrated in Figure 1.

## EXAMPLE III

293 cells and T293 cells were grown in T-25 flasks to ~90% confluence. The cells were harvested by trypsinization according to standard protocols. Briefly,

the medium was removed from the cultures and they were washed one time with 5 mL DPBS. Trypsin (1 mL of 0.05% in0.53 mM EDTA-4Na) was added to the culture for ~1 min and the flasks were rapped to dislodge the cells. Medium (4 mL) was added to stop the reaction, and the cells were pelleted by centrifugation at 176 x g for 5 min. The pellet was suspended in 5-10 mL DPBS and the centrifugation step was repeated. The cell pellet was then suspended in air drying buffer containing trehalose (10 mM Hepes, 5 mM KCL, 65 mM NaCl, 150 mM Trehalose, and 5.7% BSA with pH 7.2) at 1.4 million cells per mL. Aliquots (1.0 mL) were placed in 35 mm polysterene Petri dishes and air-dried in a ThermoForma biosafety cabinet in specific marked locations in the center of the hood over 0-24 hours. At various time points during drying, samples were removed for viability and water content analyses. Water contents were measured gravimetrically in triplicate. For viability measurements, samples were rehydrated with 1 mL medium. 50 µL of cellular suspension was mixed with 50 µL trypan blue and incubated at room temperature for 3 min. Cells were visualized at 10X by light microscopy on a hemacytometer and counted using five counts of 50-100 cells per 1 $mm^2$ hemocytometer grid square for each sample. Percent viability was calculated as the number of cells excluding the dye divided by the total number of cells. Viability was plotted as a function of water content as the means +/- standard deviation (for both variables), the results shown in Figure 2.

Example IV

293 cells and T293 cells were grown in T-25 flasks to ~90% confluence. The cells were incubated in medium with 100 mM trehalose for 24 hours at 37 °C to induce endocytotic loading. The cells were then harvested by trypsinization according to standard protocols. Briefly, the medium was removed from the cultures and they were washed one time with 5 mL DPBS. Trypsin (1 mL of 0.05% in0.53 mM EDTA-4Na) was added to the culture for ~1 min and the flasks were rapped to dislodge the cells. Medium (4 mL) was added to stop the reaction, and the cells were pelleted by centrifugation at 176 x g for 5 min. The pellet was suspended in 5-10 mL DPBS and the centrifugation step was repeated. The cell pellet was then suspended in air drying buffer containing trehalose (10 mM Hepes, 5 mM KCL, 65 mM NaCl, 150 mM Trehalose, and 5.7% BSA with pH 7.2) at 1.4 million cells per mL. Aliquots (1.0 mL) were placed in 35 mm polysterene Petri dishes and air-dried in a ThermoForma biosafety cabinet in specific marked locations in the center of the hood over 0-24 hours. At various time points during drying, samples were removed for viability and water content analyses. Water contents were measured gravimetrically in triplicate. For viability measurements, samples were rehydrated with 1 mL medium. 50 µL of cellular suspension was mixed with 50 µL trypan blue and incubated at room temperature for 3 min. Cells were visualized at 10X by light microscopy on a hemacytometer and counted using five counts of 50-100 cells per 1 $mm^2$ hemocytometer grid square for each sample. Percent viability was calculated as the number of cells excluding the dye divided by the total number of cells. Viability was plotted as a function

of water content as the means +/- standard deviation (for both variables), with the results shown in Figure 3.

## EXAMPLE V

293 cells and T293 cells were grown in T-25 flasks to ~90% confluence. The cells were incubated in medium with 100 mM trehalose for 24 hours at 37 °C to induce endocytotic loading. The cells were then harvested by trypsinization according to standard protocols. Briefly, the medium was removed from the cultures and they were washed one time with 5 mL DPBS. Trypsin (1 mL of 0.05% in0.53 mM EDTA-4Na) was added to the culture for ~1 min and the flasks were rapped to dislodge the cells. Medium (4 mL) was added to stop the reaction, and the cells were pelleted by centrifugation at 176 x g for 5 min. The pellet was suspended in 5-10 mL DPBS and the centrifugation step was repeated. The cell pellet was then suspended in air drying buffer containing trehalose(10 mM Hepes, 5 mM KCL, 65 mM NaCl, 150 mM Trehalose, and 5.7% BSA with pH 7.2) at 1.4 million cells per mL. Aliquots (1.0 mL) were placed in 35 mm polysterene Petri dishes and air-dried in a ThermoForma biosafety cabinet in specific marked locations in the center of the hood. Samples were then removed and rehydrated with 1 mL medium. Following viability testing by trypan blue exclusion (which used 50 mL), the remaining 950 mL was combined with 7 mL medium, and replated in a T-25 flask. Parallel samples were assayed for residual water content by gravimetric analysis. The cultures were incubated at 37 °C, 90% relative humidity, and 5% $CO_2$ for 24 hours, after which time the medium was removed and replaced

with fresh medium. After incubation for 6 more days under the same conditions, the medium was removed and the colonies were stained with Hema 3 and counted. The results are illustrated in Figure 4, which is a graph of the number of colonies formed after rehydration vs water content after drying the transfected 293 cells (T-293 cells) and the control 293 cells (293-cells) to 0.3 gm. water/gm. dry weight and to 0.2 gm. water/gm. dry weight, with both the transfected 293 cells and the control 293 cells having trehalose internally and with the drying buffer for both the transfected 293 cells and the control 293 cells having 150 mM trehalose.

## Conclusion

Embodiments of the present invention provide that mammalian cells (e.g., T-293 cells) transfected with the stress protein p26 and loaded with trehalose, a sugar found at high concentrations in organisms that normally survive dehydration, survived drying at water contents of about 0.5 gm water/gm dry weight cells, and below about 0.5 gm water/gm dry weight cells. Control mammalian cells not transfected with the stress protein p26, by contrast to the transfected mammalian cells, have diminished survival as early as water contents of 2 gm water/gm dry weight cells. Thus, transfection of mammalian cells with p26 and loading with trehalose improves the ability to dry mammalian cells, particularly mammalian nucleated cells.

While the present invention has been described herein with reference to particular embodiments thereof, a

latitude of modification, various changes and substitutions are intended in the foregoing disclosure, and it will be appreciated that in some instances some features of the invention will be employed without a corresponding use of other features without departing from the scope and spirit of the invention as set forth. Therefore, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope and spirit of the present invention. It is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments and equivalents falling within the scope of the appended claims.

What Is Claimed Is:

1. A method for increasing the survival of a biological sample comprising:

providing a biological sample having a stress protein;

loading the biological sample with a carbohydrate to produce a loaded biological sample; and

dehydrating the loaded biological sample while maintaining a residual water content in the biological sample of at least about 0.01 g water per gram of dry weight of biological sample to increase survival of the biological sample.

2. The method of Claim 1 additionally comprising

storing the dehydrated loaded biological sample to produce a stored biological sample; and

rehydrating the stored biological sample.

3. The method of Claim 1 wherein said biological sample comprises a mammalian biological sample.

4. The method of Claim 1 wherein said providing a biological sample having a stress protein comprises transfecting the biological sample with the stress protein.

5. The method of Claim 1 wherein said providing a biological sample having a stress protein comprises loading

the biological sample with the stress protein from a solution having the stress protein.

6. The method of Claim 1 wherein said stress protein comprises p26.

7. The method of Claim 1 wherein said loading of the biological sample with a carbohydrate comprises disposing a biological sample in a solution having the carbohydrate and a temperature of at least $25^0$ C for transferring the carbohydrate from the solution into the biological sample.

8. The method of Claim 7 wherein said transferring the carbohydrate is by fluid phase endocytosis.

9. A method for increasing the survival of a biological sample comprising:

providing a biological sample having a stress protein; and

dehydrating the biological sample while maintaining a residual water content in the biological sample of at least about 0.01 g water per gram of dry weight of biological sample to increase survival of the biological sample.

10. A biological sample produced in accordance with the method of Claim 1.

11. A method for proliferating biological samples comprising:

providing biological samples having a stress protein;

dehydrating the biological samples;

rehydrating the biological samples to produce rehydrated biological samples; and

proliferating the rehydrated biological samples.

12. The method of Claim 11 additionally comprising loading the biological samples with a carbohydrate.

13. The method of Claim 11 wherein said dehydrating the biological samples comprises maintaining a residual water content in the biological sample of at least about 0.01 g water per gram of dry weight of biological samples to increase survival of the biological samples.

14. The method of Claim 13 additionally comprising storing, prior to rehydrating the biological samples, the dehydrated biological samples.

15. A dehydrated mammalian biological sample comprising at least one protein having at least about 0.05 % by weight of a stress protein; and at least about 5 mM of a carbohydrate.

16. The dehydrated mammalian biological sample of Claim 15 additionally comprising at least about 0.01 g water per gram of dry weight of biological sample.

17. A method for preparing a biological sample for treatment comprising:

providing a biological sample; and

inserting into the biological sample cDNA for p26 cloned from *Artemia franciscana* so the biological sample produces p26.

18. The method of Claim 17 wherein said biological sample comprises a eukaryotic cell.

19. A biological sample produced in accordance with the method of Claim 18.

## Abstract of the Disclosure

A dehydrated composition is provided that includes dried biological samples containing a stress protein and a solute. A method for increasing the survival of biological samples comprising providing biological samples having a stress protein (e.g., p26), loading the biological samples with a carbohydrate to produce loaded biological samples, and dehydrating the loaded biological samples while maintaining a residual water content in the biological samples of at least about 0.01 gram water per gram of dry weight of biological samples to increase survival of the biological samples upon rehydrating.

Figure 1:

In this figure, it can be seen that transfected cells (T-293) and the control (293) cells survive drying in the absence of trehalose.

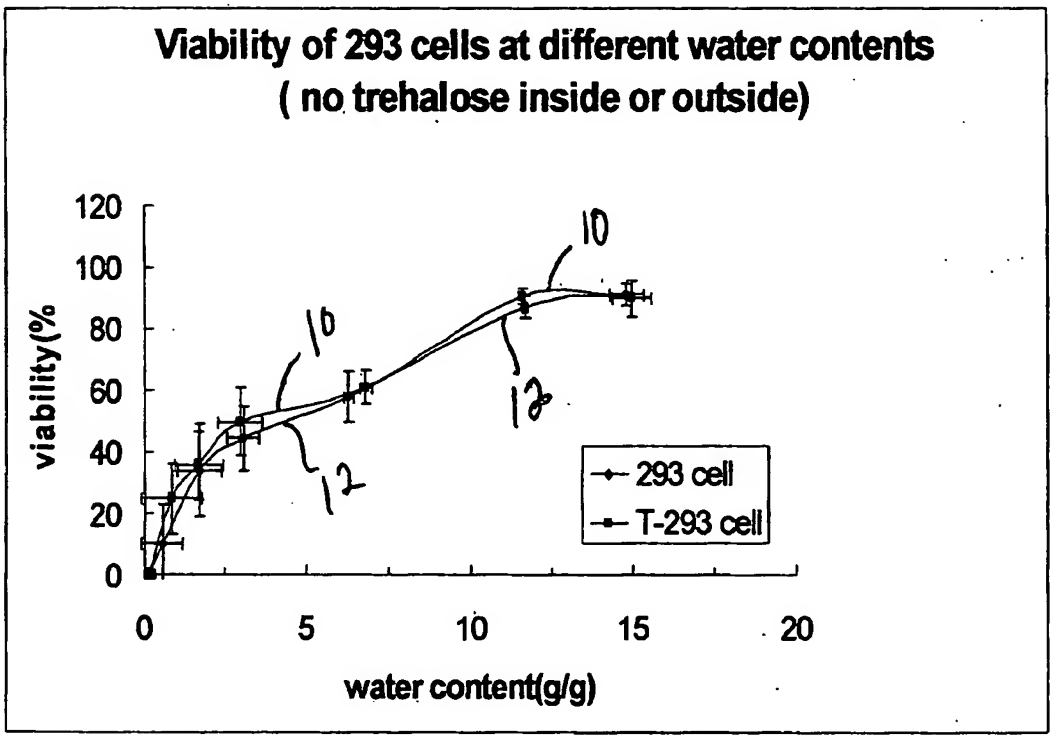


Fig. 1

Figure 2

Transfection of the cells (T-293) improves slightly survival when cells are dried with trehalose outside only.

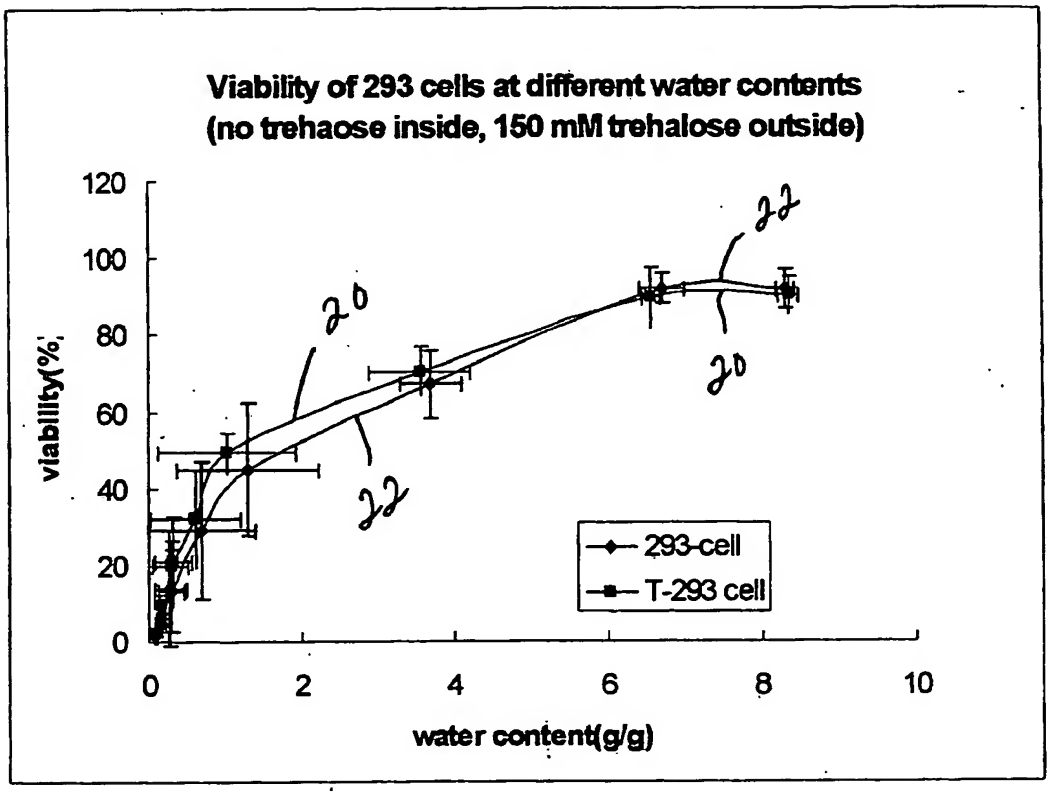


Fig 2

Figure 3:

Transfected cells (T-293) loaded with trehalose and dried with trehalose outside as well, show greatly improved viability when compared with control non-transfected (293) cells.

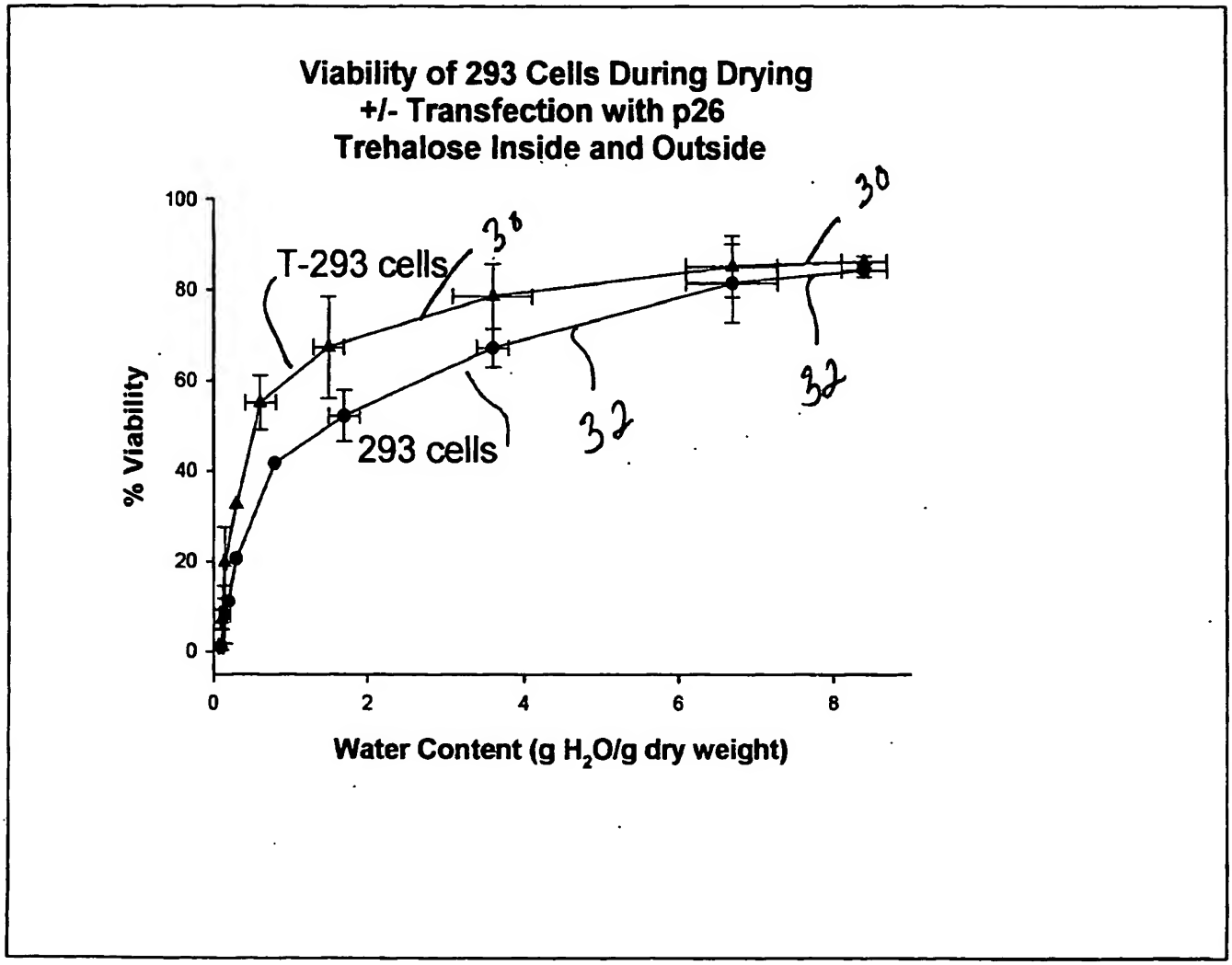


Fig 3

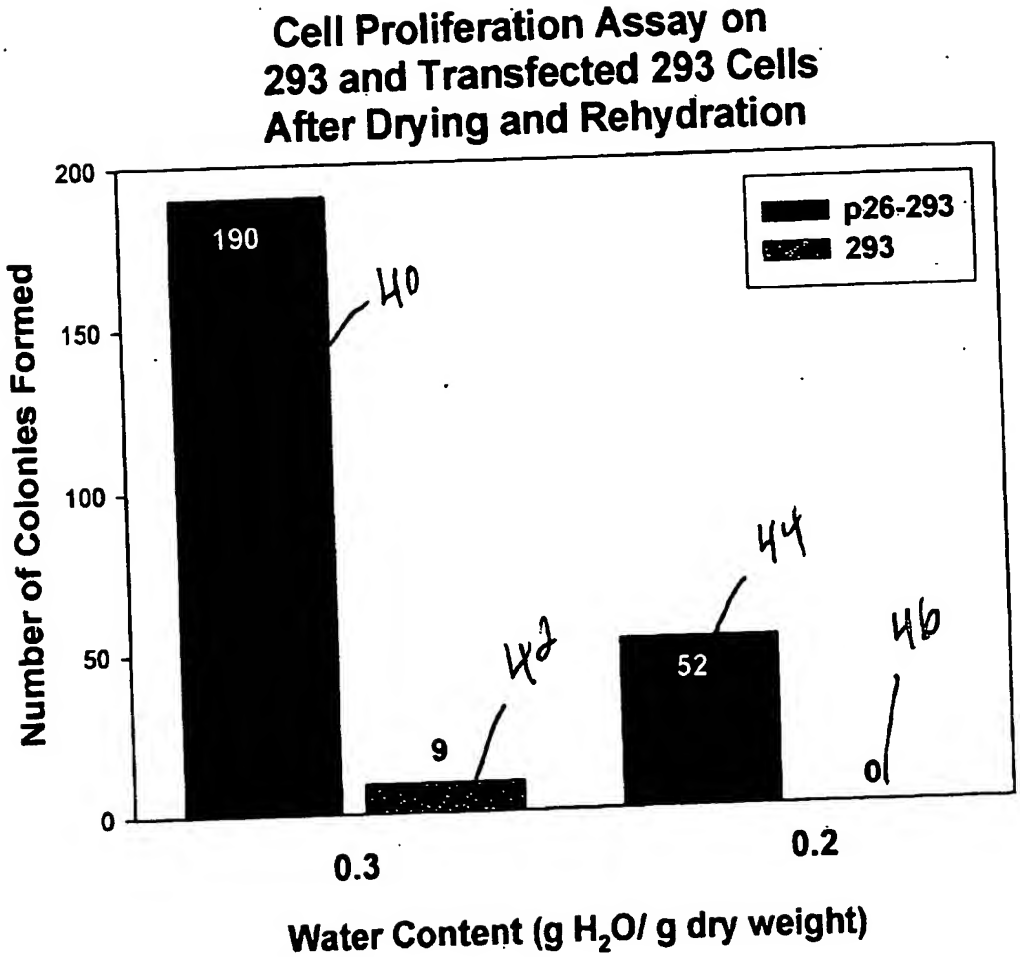


Fig. 4

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